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SYNTHESIS AND IN VITRO CYTOTOXICITY OF CRYPTOPHYCINS AND RELATED ANALOGS

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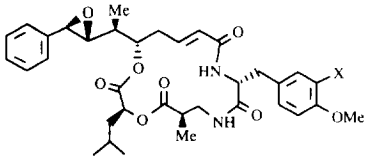
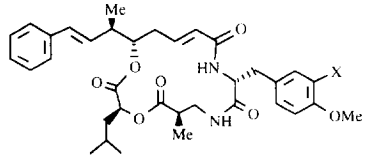
Abstract: Several members of the Cryptophycin family were synthesised using a straightforward convergent approach. The proposed synthetic route was used to prepare novel analogs of Cryptophycins A and B in which the benzylic epoxide moiety was replaced by alternate electrophilic functions. The effect of these modifications on cytotoxic activity was determined on several tumor cell lines.

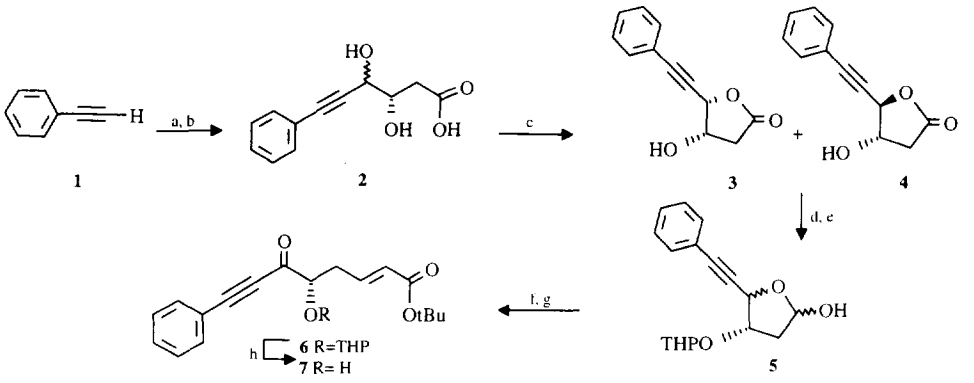


X = Cl : **Cryptophycin A**
X = H : **Cryptophycin B**



X = Cl : **Cryptophycin C**
X = H : **Cryptophycin D**

In 1994, Moore and co-workers isolated from the blue-green alga (cyanobacterium) *Nostoc* sp. GSV 224, a series of metabolites known as Cryptophycins.[1] These compounds displayed very potent in vitro cytotoxicity against several human tumor cell lines and experimental evidence shows that the effect is due to irreversible inhibition of tubulin polymerisation into microtubules.[2] Both in vitro [1] and in vivo [3] data suggest that the presence of the benzylic epoxide moiety is very important for biological activity. For instance, Cryptophycin A shows a much better in vitro profile than its deoxy counterpart Cryptophycin C in the KB (human nasopharyngeal cell line)(IC_{50} A: 5 pg/ml; IC_{50} C: 3000 pg/ml).[1] The in vivo studies follow the same trend but despite its very high in vitro potency, Cryptophycin A displays very good activity only at relatively high doses (30-132 mg/kg).[3] One of the possible reasons for this poor translation may be the inherent instability of the benzylic epoxide that might rapidly degrade into inactive compounds. In order to solve this problem while preserving the electrophilic character of the benzylic position; the epoxide was replaced by other functionalities including enones, ynones, as well as allylic and propargylic electrophiles. The retrosynthetic analysis led to four fragments: D-tyrosine, 2-hydroxy-isocaproic acid, 3-amino-2-methyl propionic acid and a phenyl octanoic acid derivative.[4] The first two fragments are commercially available while the β-amino acid unit was easily prepared from (S)-(+)-3-bromo-2-methyl-1-propanol.[5]

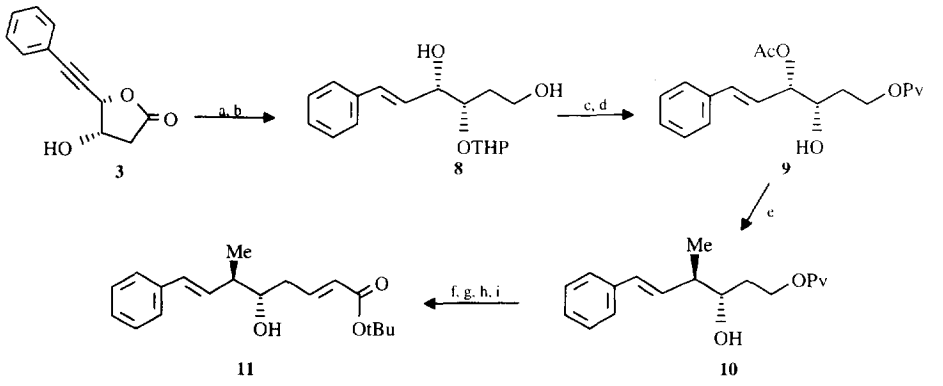


Scheme 1

(a) BuLi, THF, -78 °C; then (S)-(-)-2-Acetoxysuccinic anhydride; (b) $NaBH_4$, EtOH; then 1N NaOH, 0 °C; (c) PTSA, benzene, 50 °C,63% from **1**; (d) Dihydropyran, PTSA, THF, 0 °C to rt; (e) DIBAL-H, toluene -78 °C; (f) (tert-butoxycarbonylmethylene)-triphenylphosphorane, THF, 50 °C; (g) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -78 °C to 0 °C, (87% from **4**); (h) AcOH, H_2O, rt, 70%.

The synthesis of the octanoic acid fragment with an ynone as electrophilic functionality was realized in 7 practical steps (Scheme 1). Phenylacetylene (**1**) was treated with n-butyllithium and added to a solution of (S)-(-)-2-acetoxysuccinic anhydride[6] in tetrahydrofuran at -78 °C. After 10 min, sodium borohydride was added, followed by an aqueous solution of sodium hydroxide, to produce a diastereomeric mixture of carboxylic acids **2**. Interestingly, the anhydride opening was regioselective,[7] whereas the ketone reduction led a 1:1 mixture of both isomers. Heating this mixture with *p*-toluenesulfonic acid (PTSA) in benzene at 50 °C, afforded butyrolactones **3** and **4**,[8] easily separated by flash chromatography, in 63% combined yield from **1**. Both lactones could be used to complete the synthesis of the octanoic moiety. For example, **4** was treated with dihydropyran and a catalytic amount of PTSA in THF, followed by partial reduction with DIBAL-H in toluene at -78 °C to give the corresponding mixture of lactols **5**. The latter was then treated with (tert-butoxycarbonylmethylene)-triphenylphosphorane and the resulting alcohol was submitted to Swern oxidation affording **6** in 87% overall yield from **4**. The tetrahydropyranyl group was then cleaved in acetic acid and water to give the desired octanoic derivative **7** in 70% yield. The octadienoic ester fragment required for the synthesis of the natural products was obtained from the butyrolactone **3** (Scheme 2). Protection of the free alcohol with dihydropyran and PTSA, in tetrahydrofuran, followed by reduction with lithium aluminium hydride (LAH) in ether gave diol **8** in 92% overall yield. The primary and secondary hydroxyls were sequentially esterified with pivaloyl chloride and acetic anhydride respectively in 59% overall yield. The dimethyl cuprate reaction on the fully protected compound failed and led to a mixture of conjugated dienes, probably by a reduction-elimination mechanism. In order to avoid this process, the tetrahydropyranyl group was cleaved and the reaction was carried out on **9**. In this case, the reaction produced a 1:1 mixture of SN^2 and $SN^{2'}$ products. The desired product **10** was isolated by flash chromatography in 34% yield. The secondary alcohol was then reprotected and the pivaloate group was cleaved with LAH. Swern oxidation of

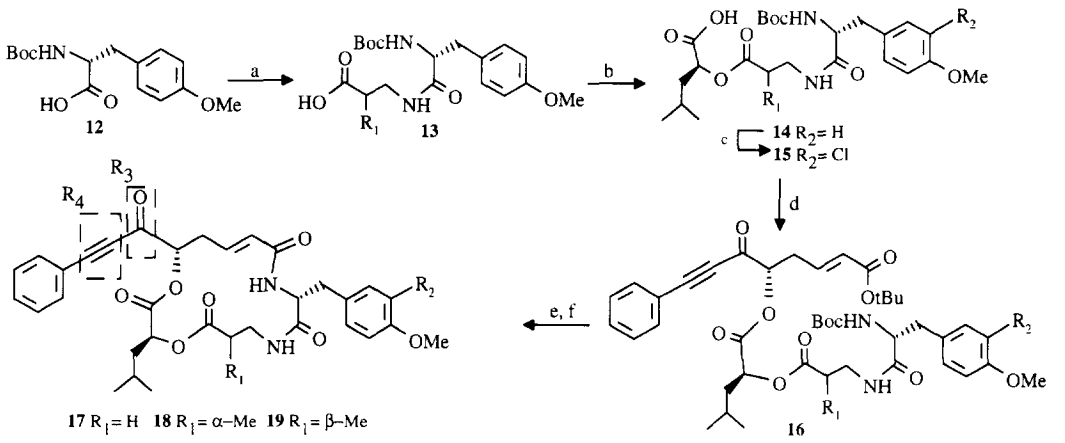
the resulting alcohol followed by reaction with (tert-butoxycarbonylmethylene)-triphenylphosphorane afforded, after a treatment with acetic acid and water, the desired alcohol **11** in 58% overall yield from **10**.



Scheme 2

(a) DHP, PTSA, THF, rt, 92%; (b) LAH, Et_2O, rt, 100 %; (c) PvCl, Pyr., DMAP, 0 °C to rt; then Ac_2O, 59%; (d) AcOH, H_2O, 100%; (e) $(Me)_2CuLi$, Et_2O, 0 °C, 34% ; (f) DHP, PTSA, THF, r.t., 98%; (g) LAH, THF, 0 °C, 97%; (h) $(COCl)_2$, DMSO, Et_3N, CH_2Cl_2, -78 °C to 0 °C; then (tert-butoxycarbonylmethylene)-triphenylphosphorane, 73%; (i) AcOH, H_2O, 40 °C, 80%.

Having the four fragments in hand, synthesis of the macrocyclic structures was undertaken (Scheme 3). N-protected O-Methyl D-tyrosine **12** was treated with dicyclohexylcarbodiimide (DCC) and N-hydroxysuccinimide in dry dimethoxyethane at 0 °C.[9] The required β-amino acids and triethylamine in water were then added to the reaction mixture and after work up, the coupling products **13** were obtained in 80-93% yields. The acids **13** were treated with 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCI) and N-hydroxysuccinimide in dimethoxyethane and dichloromethane. The activated esters were then reacted with (S)-(-)-2-hydroxy-isocaproic acid and dimethylaminopyridine (DMAP) in acetonitrile to produce **14** in 80-90% yield after purification. The chlorine atom on the aromatic ring of the tyrosine portion of Cryptophycins A and C was introduced on **14** by a treament with sulfuryl chloride in acetic acid,[4] followed by BOC-ON and triethylamine to reprotect the amino group. The chloro derivative **15** (with R_1 = β-Me) was obtained in 68% yield from **14**. In the case of **17z''**, the chlorine atom was introducted directly on the macrocyclic compound **17k**. Coupling of **7** with the bottom fragments were carried out with trichlorobenzoyl chloride, triethylamine and DMAP[10] to produce **16** in 68-84% yields. The protecting groups were then cleaved by a treatment with trifluoroacetic acid in dichloromethane and the resulting amino acids were cyclized with O-benzotriazol-1-yl-N,N,N',N', -bis(pentamethylene) uronium hexafluorophosphate[11] and diisopropylethylamine, in acetonitrile or DMF, at 0 °C. The macrocyclic structures **17a**, **18a** or **19a** were obtained in 50-70% yield from **16**. The R_3 and R_4 portions of these molecules could then be modified using standard chemical transformations giving access to several original derivatives (Table 1).



Scheme 3

(a) DCC, N-hydroxysuccinimide, DME, 0 °C; then $HO_2CCHR_1CH_2NH_2$ (R_1= H, Me), Et_3N, H_2O, rt, 80-93%; (b) EDCI, N-hydroxysuccinimide, DME, CH_2Cl_2; then (S)-(-) -2-hydroxy-isocaproic acid, DMAP, CH_3CN, rt, 80-90%; (c) SO_2Cl_2, AcOH, 55°C; then BOC-ON, Et_3N, CH_2Cl_2, 68%; (d) Trichlorobenzoyl chloride, Et_3N, THF, rt; then **7**, DMAP, Et_3N, Toluene, rt, 68 -84%; (e) TFA, CH_2Cl_2; (f) O-Benzotriazol-1-yl -N,N,N',N',-bis(pentamethylene) uronium hexafluorophosphate, DIPEA, CH_3CN or DMF, 0 °C, 50-70% from **16**.

Table I

	R_2	R_3	R_4		R_2	R_3	R_4
a	H	=O	triple bond	**o**	H	α or β OH	epoxide
b	H	=O	cis double bond	**p**	H	H_2	trans double bond
c	H	=O	trans double bond	**q**	Cl	H_2	trans double bond
d	H	=O	$-CH_2CH_2-$	**r**	H	α–H, β–Me	trans double bond
e	Cl	=O	trans double bond	**s**	Cl	α–H, β–Me	trans double bond
f	H	α or β OMs	triple bond	**t**	H	α–H, β–Me	epoxide
g	H	α or β Br	triple bond	**u**	Cl	α–H, β–Me	epoxide
h	H	α–OH, β–Me	triple bond	**v**	H	α or β OAc	trans double bond
i	H	β–OH, α–Me	triple bond	**w**	H	α or β OH	trans double bond
j	H	α or β OH	triple bond	**x**	H	β–H, α–Me	trans double bond
k	H	H_2	$-CH_2CH_2-$	**y**	H	β–H, α–Me	epoxide
l	H	α or β OH	$-CH_2CH_2-$	**z**	H	H_2	epoxide
m	H	β–OH, α–Me	cis double bond	**z'**	H	H_2	triple bond
n	H	α–OH, β–Me	cis double bond	**z''**	Cl	H_2	$-CH_2CH_2-$

For comparison purposes, the natural products were prepared using the same strategy from **14** or **15** (R_1= β–Me) and **11** as the upper fragment. Cryptophycins and related analogs were evaluated for their cytotoxic activity against B16F10 mouse melanoma cells, DA-3 mouse mammary adenocarcinoma cells, KB human nasopharyngeal carcinoma cells, KBV vinblastine resistant KB cells and HT-29 human colon adenocarcinoma cells (Table II).

Table II. Cytotoxicity (IC_{50}(ηM)) of Compounds **17**, **18** and **19** in MTT assay.[12]

	B16F10	DA-3	KB	KBV	HT-29		B16F10	DA-3	KB	KBV	HT-29
17a	>10	>10	>10	>10	>10	**18z'**	>10	>10	>10	>10	>10
17b	>10	>10	>10	>10	>10	**19a**	>10	>10	>10	>10	>10
17c	>10	>10	>10	>10	>10	**19c**	>10	>10	>10	>10	>10
17d	>10	>10	>10	>10	>10	**19e**	>10	>10	>10	>10	>10
17f	>10	>10	>10	>10	>10	**19f**	>10	>10	>10	>10	>10
17g	>10	>10	>10	>10	>10	**19g**	>10	>10	>10	>10	>10
17h	>10	>10	>10	>10	>10	**19l**	>10	>10	>10	>10	>10
17i	>10	>10	>10	>10	>10	**19o**	>10	>10	>10	>10	>10
17j	>10	>10	>10	>10	>10	**19p**	>10	>10	>10	>10	>10
17k	>10	>10	>10	>10	>10	**19q**	>10	>10	>10	>10	>10
17l	>10	>10	>10	>10	>10	**19r**	>10	>10	>10	>10	>10
17m	>10	>10	>10	>10	>10	**19s**	2	1	2	5	1
17n	>10	>10	>10	>10	>10	**19t***	0.3	0.2	0.3	0.8	0.3
17w	>10	>10	>10	>10	>10	**19u***	0.03	0.002	0.004	0.035	0.002
17z''	>10	>10	>10	>10	>10	**19v**	>10	>10	>10	>10	>10
18a	>10	>10	>10	>10	>10	**19w**	>10	>10	>10	>10	>10
18f	>10	>10	>10	>10	>10	**19x**	>10	>10	>10	>10	>10
18g	>10	>10	>10	>10	>10	**19y***	4	>10	>10	>10	3
18j	>10	>10	>10	>10	>10	**19z***	>10	3	6	>10	4

* Tested as an isomeric mixture of epoxides.

All attemps to modify the side chain of the natural products (**19r-u**) led to inactive or marginally active (2-5 μM) compounds. **19y** (α–Me in the side chain) **19z** (absence of methyl group in the side chain) show that the presence of the β-methyl group at the allylic position of the side chain is very important for a good biological activity. Consequently, the electrophilic character of the benzylic position is not the only factor responsible for the biological activity.

In conclusion, a practical synthesis of Cryptophycins and related analogs was developed. More than 30 analogs were tested on 5 different tumor cell lines. The side chains of the natural products play a crucial role in the biological activity.

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